Anti-thrombocytopenic activity of *Amaranthus spinosus* in ethanol-induced rat models exhibiting low platelet count

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Abstract: Thrombocytopenia is a condition manifesting abnormally low platelet count in the body. Current treatment for this condition involves the use of expensive recombinant thrombopoetin and interleukin-11 to stimulate platelet production. Platelet transfusions have also been used. Clearly, there is still a need for more alternative but affordable and convenient treatment for this condition. The increasing platelet effects of the fresh decoction of *Amaranthus spinosus* were investigated in Sprague-Dawley rats with decreased platelet counts induced by ethanol. The crude extract was used to determine the total phenolics for the assessment of the plant's antioxidant activity. Using UV-Vis spectrophotometry, the result revealed the presence of the phenolics.The anti-thrombocytopenic activity of *A. spinosus* was evaluated by platelet count, bleeding time and clotting time in four groups. Group A served as the treated group given 100mg/kg of *A. spinosus*. Group B served as the positive control group given intraperitoneal ethanol at 3g/kg body weight. Group C and D were identified as the vehicle and time control groups, respectively. Results revealed a significant increase in the platelet count and decrease in bleeding and clotting time in the ethanol-induced thrombocytopenic rats treated with *A. spinosus* for 14 days ($p<0.05$). Furthermore, the comparisons of the histopathological analysis of all groups revealed the decreased sinusoidal dilation and preventive effect of hepatic necrosis in treated group. Hence, the possible use of *A. spinosus* as an antithrombocytopenic decoction is attributable to its influence on its hepatocurative property and probably to the platelet protecting activity of its antioxidant polyphenolic constituents.

Keywords: *Amaranthus spinosus*, thrombocytopenia, platelet, bleeding time, clotting time, polyphenolics

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INTRODUCTION

Thrombocytopenia, leukopenia and hemoconcentration are constant findings in patients infected with dengue virus (Bomasang & Masaga, 2008; Gulati & Maheshwari, 2007). Thrombocytopenia (TCP) is a condition wherein there is an abnormally low platelet count in the body (usually less than 150 x 10^9/L). It is caused by sequestration of platelets in an enlarged spleen, impaired platelet production, and immune and non-immune mediated platelet destruction (Kujovich, 2005). Chronic liver can cause this condition as well as from intake of various drugs such as alcohol, chloramphenicol, heparin and cancer chemotherapeutic drugs (Apostol et al., 2012). Dengue hemorrhagic fever which is the most serious form of dengue manifests thrombocytopenia. The relevance of thrombocytopenia in the individual patient is variable and depends on the clinical presentation. Because platelets play an essential role in preserving vessel wall integrity, thrombocytopenia is associated with a defect of primary hemostasis (Stasi, 2012). Among 1468 drugs suspected of causing thrombocytopenia, 23 of these drugs had showed an evidence for an association with the aforementioned disease (Reese et al., 2011). Stimulation of platelet production is the current treatment for this condition such as the use of expensive interleukin-11 and recombinant thrombopoetin. Only limited efficacy can be obtained from platelet transfusion which has been also used as a treatment. Clearly there is still a need for more alternative but affordable and convenient cure (Apostol et al.,2012).

Amaranthus spinosus, seen in Figure. 1, is usually spread throughout the tropics and warm temperate regions of Asia (Mishra, Verma, Mukerjee, & Vijayakumar, 2012). *A. spinosus* is commonly named in the Philippines as Uray (Tagalog), Ayantoto (Pampanga), Kolitis (Bisaya), Kalunay (Ilokano), Alayon (Ifugao) and Akum (Maguindanao). In English, it is called the Spiny amaranth, Thony amaranth, Pigweed, Needle burr, Thorny pigweed, Calaloo and Prickly calau. It has a brown surface, but the inside is cream in color. The root is about 10 to 12 cm in length and 0.1 to 0.3 mm in width, and it easily

Figure 1. *Amaranthus spinosus* (Uray)

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breakable by hand. It has a pleasant odor and has a slightly sweetish taste (Jhade, Ahiwar, Jain, Sharma, & Gupta, 2011). All parts of the plant can be utilized for medicinal purposes(Garcia et al., 2003). Phenolics are one of the most various groups of phytochemicals that entirely distributed in fruits, vegetables and herbs. The extraction of these compounds can be influenced by various parameters such as conditions, solvent polarity, particle size, and extraction procedures (Luthria, Mukhopadhyay & Kwansa, 2006). Several active phenolic that can be found in *A.spinosus* are alkaloids, flavonoids, and glycosides. Other medically important components are the phenolic acids, saponins, amino acids, linoleic acid, lipids, terpenoids, steroids, b-sitosterol, stigmasterol, rutin, catechuic tannins and carotenoids (Jhade, Ahiwar, Jain, Sharma, & Gupta, 2011). There are also some identified betalains in the stem bark of like hydroxycinnamates quercetin ,amaranthin, isoamaranthine, rutin and kaempferol glycosides (Stinzing et al., 2004). It also contains coumaroyl adenosine along with stigmasterol, glycoside amaranthoside, lignan glycoside and amaricin. Betalains have a lot of benefits as a phenolic constituent because it is well known for their anticancer, antioxidant, antiviral and antiparasitosis properties (Dai & Mumper, 2010; Kumar, Lakshman, Jayaveera, Khan, Manoj, & Swamy, 2010; Mathur, Khatri, Samanta, Sharma, & Mandal, 2010; Srivastava, Singh, Gul, & Ahirwar, 2011). The leaves and roots have been used for application by poultice to relief bruises, burns, abscesses, menorrhagia, and gonorrhoea (Vardhana, 2011). Previous researches revealed that it was scientifically described for analgesic properties, antifertility activity, anti-inflammatory activity, antiprotozoal activity, anti-malarial activity, immuno-modulatory properties, anti-hyperlipidemic and spermatogenic effects, and traditionally it is used to cure wounds, jaundice and to prevent swelling around stomach (Baral, Chakraborty,& Chakraborty, 2010; Jhade, Ahiwar, Jain, Sharma, & Gupta, 2011). Antioxidants have important role in prevention and treatment of disorders and plants are considered as rich sources of radical scavenging compounds (Hajimedipoor, Adib, Khanavi, Mobli, Amin & Moghadam 2012). *A. spinosus* antioxidant activity was evidently caused by its phytochemical componentssuch as the flavonoids (Kumar, Lakshman, & Jayaveera, 2008). It was also suggested that *Amaranthus spinosus* has an antibacterial and antimicrobial properties that can be used as new drugs for the therapy of infectious diseases (Vardhana, 2011). In the traditional systems of medicine, the whole plant is used as food additives and for the treatment of diarrhea and ulcer. The antidiarrheal activity of the extract of *A. spinosus* is due to the inhibitory effects on both gastrointestinal propulsion and fluid secretion caused by the presence of phenolics, tannins, and flavonoids. Its free radical

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scavenging activity was used as conformation for the use in traditional medicine. The stem extract and water extract of the plant showed antimalarial activities and significant immunostimulating activity respectively (Hilou, Nacoulma, & Guiguemde, 2006; Hussain, Amresh, Singh & Rao, 2009; Sarin, & Bafna, 2012). The water extract indeed inhibited the dexamethasone-induced apoptosis of murine primary splenocytes. The suggested component that was responsible for that activity is its high molecular weight, and its heat-labile component (Lin, Chiang & Lin, 2004; Lin, Li, & Lin, 2008) anthraquinone derivatives content have induced increase in gastrointestinal motility (Kumar, Lakshma,& Jayaveera, 2008). The plant is said to possess spasmolytic effect and bronchodilator activity which explains its traditional uses in airways disorders (Chaudhary et al., 2012). The hepatoprotective activity of the plant is due to its ability to elevate enzymatic levels of serum glutamate pyruvate transaminase (ALT), serum glutamate oxaloacetate transaminase (AST), and serum alkaline phosphatase (SALP) which may be responsible for glycogen and protein increase by its involvement in protein and enzyme synthesis, total bilirubin, glycogen metabolism and in the transportation of metabolites across the cell membrane. Thus, it is considered to be safe for the treatment in liver problems at normal therapeutic doses (Gul, Srivastava, & Ahirwar, 2011). The plant is also safe to be used against various types of blood related disorders (Srinivasa et al., 2010). Adequate precaution should be observed when this plant is used in animals to avoid any probable toxic effects (Olufemi, Assiak, Ayoade, & Onigemo, 2003).

Liver damage caused by chronic administration of ethanol will lead to an increase in the blood pressure of the portal veins. The impaired liver function impedes the outflow of blood from the spleen. This results to platelet sequestering in the spleen.Sinusoidal dilation of the aforementioned organs are often the consequence of impaired venous outflow (Kakar, Kamath & Burgart, 2004).

Alcohols are said to be good platelet inhibitors (Ruf, 2004). A number of studies have showed that ethanol directly affects hemostasis via a number of mechanisms including platelet function (Salem & Laposata, 2005). Alteration of the platelet distribution by the chronic use of ethanol has been known to cause thrombocytopenia. Ethanol was used in this study for the induction of thrombocytopenia because it is one of the most common substances used worldwide with many commercial and industrial applications (Apostol, et al., 2012).

This study was done to determine the efficacy of the platelet-increasing effects of *A. spinosus* in ethanol-induced thrombocytopenic rats. The platelet count, bleeding time, and clotting time were used to assess the effectiveness of the plant extract. Histopathological analysis

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of the liver and spleen was conducted to achieve further evidence of the mechanism of *A. spinosus*in thrombocytopenia.This research aims to secure evidence for the use of as an alternative form of treatment for disease and drug- induced thrombocytopenia.

MATERIALS AND METHODS

Plant Collection and Authentication

The plantwas collected from its innate habitat in San Pascual Batangas, Philippines. The sample submitted was identified and authenticated by Mrs. Priscilla Barcial, MSC who is a weed specialist from University of the Philippines Los Baños.

Preparation of decoction

The selection of solvent and form of plant material was made to contend with the traditional way of preparing herbal treatments by the native Filipinos. The decoction was derived from washing the adhering soil and debris of the whole plant (100 grams) and boiling it in 500 mL of distilled water for 15 minutes at a temperature of 100°C. It was stored in steriled glass vials. Fresh decoction was prepared and induced every morning during the 14 days period of treatment. The decoction appeared as pale yellow green liquid.

Determination of Total Phenolic Content

Ground fresh plant sample was mixed with 95% ethanol in the dark at 25ºC overnight. It was shaken during the extraction time to ensure complete extraction. The extracts were filtered through Whatman No. 4 paper. Ethanol was evaporated using a steamer. The evaporated plant extracts were about 2 grams of brown sticky liquid in glass dish.

The plant extract was submitted to the Industrial Technology Development Institute Standards and Testing Division at DOST. Total phenolics were measured with Gallic Acid by means of UV-Vis spectrophotometry.

Reagent

The reagent used was the 95% ethanol (Tradewell, India) which was purchased from DKL (Phillipines).

Test animals

Before the procurement of the animal subjects, the protocol was submitted to the Ethics Committee of the Lyceum of the Philippines University- Batangas for approval.

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The rats were purchased from the University of the Philippines Manila-Department of Pharmacology. The male Sprague-Dawley rats, weighing 150-200 grams, were housed for acclimation in the animal room of Lyceum of the Philippines University. The lighting sequence (12 hrs. light and 12 hrs. dark), relative humidity (55 +/- 5%), and temperature (22-25ºC) were the conditions employed while the animals were housed to ensure that the standards were followed. The rats were fed using a standard rat pellet diet. They were given free access to distilled water that was replenished daily along with the supply of food. The rats were handled in correspondence with the protocols accepted by the institution and committee in charge.

Preparation of Laboratory Test Animals (Apostol et al., 2012)

A total of 16 Sprague- Dawley rats were used. They were divided into 4 equal groups (A,B,C and D) consisting of 4 rats per group (Mustapha et al., 2011). Group A, the test group, was receiving the *A.spinosus* decoction. Group B,the positive control, only received ethanol. Group C was given an identical volume of water (distilled) during the *A.spinosus* administration and this group served as the vehicle control. Lastly, group D functioned as the time control. It did not undergo any treatment during the course of the experiment.

Group A	Test Group (Ethanol induced and *A. spinosus treated*)
Group B	Positive Control (Ethanol induced only)
Group C	Vehicle Control (Water during plant administration)
Group D	Time Control (no treatment)

Platelet count determination

The retro-orbital sinus bleeding technique was the method employed for platelet determination. The method required anesthesia because of its invasiveness and it was assisted and monitored by veterinarian (Hashemiet al., 2007). Platelet count was determined through the use 0.5 ml of blood collected in EDTA tubes along with the use of the *Newbauer hemocytometer and ammonium oxalate as the diluting fluid*. This was processed in Rite Lab and Polyclinic. There were 4 blood collections conducted during the course of the experiment: before the induction of thrombocytopenia through the use of ethanol (day 0), 7 days after decreasing the platelet count (day 7), 7 days after administration (day 14), and 14 days after administration (day21) (Apostol et al., 2012).

Bleeding time determination

Using Duke's method, bleeding time was determined. An incision was done to the tail with about 4 mm deep until blood comes

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out. The bleeding tail was clotted on a #4 Whatman filter paper until no more blood was seen on the filter paper. The time from first application to the disappearance of blood was recorded. This procedure was replicated every blood collection (Apostol et al., 2012).

Clotting time determination

Using slide method, a drop of blood was placed onto a slide and was rubbed using a lancet until fibrin threads were seen. The time from the first contact with the lancet and the formation of threads were recorded. Repetition of the procedure were done every blood collection (Apostol et al., 2012).

Induction of thrombocytopenia

Group A and B were employed in ethanol-induced thrombocytopenia. For seven days, the groups were induced intraperitoneally with ethanol (3g/kg body weight) (Apostol et al., 2012).

***Amaranthus spinosus* treatment**

After the administration of ethanol-induced thrombocytopenia, the test group (A) was given decoction at a dose of 100 mg/kg body weight orally. Blood collections were done on the 7^{th} and 14^{th} days of the treatment. The decoction was given for 14 days (Apostol et al., 2012).

Histopathologic exam

After relevant procedures, the test animals were sacrificed using chloroform. A slide preparation of the liver and spleen was made by the Pathologists Diagnostic Center. Group comparisons were made between all four groups.

Statistical treatment of data

All statistical analyses were performed at a 0.05 level of significance using PASWS ver. 18. For the verication of a significant decrease of platelet count, increase bleeding time and clotting time after induction of ethanol, a paired t-test was used. F- test with Tukey's HSD test as a post-hoc procedure was used to determine if there exist significant differences in the treated, time and vehicle control groups during the course of the treatment phase (Apostol et al., 2012).

RESULTS AND DISCUSSION

I. Total Phenolic Content

The plant extract was submitted to the Industrial Technology Development Institute Standards and Testing Division at DOST. Total

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phenolics were measured as to Gallic Acid using UV-Vis spectrophotometry. The result of the antioxidant activity (total phenolics as gallic acid, %w/w) is 2.16. This pertains to the presence of phenolics in that has excellent antioxidant properties. The influence of is parallel to the presentation of antioxidants to counter platelet oxidation and platelet dysfunction. Platelet function and lifespan are maintained by reducing
the platelet oxidation, thus improving platelet count, bleeding and clotting times (Apostol, Gan, Raynes, Sabado, Carigna, Santiago & Ysrael, 2012).

II. Haematological Parameters

Table 1 shows the results of the plateletcount, bleeding time and clotting time of all the four groups based on their mean value. It can be seen that the first blood collection values were used as the baseline during the experiment. Upon the induction of ethanol (2nd blood collection), the platelet count of the test group (562.5 to 321.75) and positive control group (530.5 to 315.75) generally decreased while the bleeding and clotting time were prolonged. This is may be due to ethanol which has been known to lower the platelet count by altering platelet distribution (Apostol et al., 2012).

Table 1
Results of Haematological Parameters in all blood collection expressed in mean

	Platelet Count (x10^9/L)			
	1st	**2nd**	**3rd**	**4th**
Group A	562.5	321.75	556.25	677.5
Group B	530.5	315.75	550	503
Group C	446.25	458	550.5	593.5
Group D	408	417	513	640.5
	Bleeding time (seconds)			
	1st	**2nd**	**3rd**	**4th**
Group A	90	127.5	82.5	60
Group B	157.5	232.5	195	135
Group C	112.5	97.5	90	105
Group D	142.5	150	150	127.5
	Clotting time (seconds)			
	1st	**2nd**	**3rd**	**4th**
Group A	43	106	45	31.75
Group B	47	120	40	90
Group C	44.25	52.75	52	60
Group D	45.25	43.25	45	38.25

Blood Collection:
1st - Before induction of ethanol (Baseline)
2nd - Ethanol Inducays tion for 7 days
3rd - Treatment with *A. spinosus* for 7 days
4th - Treatment with *A. spinosus* for 14 d

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There is only a slight elevation in the platelet count of vehicle control and time control which appeared to be normal because no induction had occured to them. The bleeding and clotting time of these two groups were a bit close to their baseline compared to test and positive control groups which indicates they are still within their normal value. During the 3rd blood collection, test group was already treated with *A. spinosus* for 7 days. The platelet count of this group generally increased (321.75 to 556.25) while the bleeding (127.5 to 82.5) and clotting time (106 to 45)were reduced. Positive control group only showed slight elevation in the platelet count (315.75 to 550) which may indicate that body is trying to compensate or naturally responding to the reduction which was done by the ethanol. Vehicle and time control group values generally slightly elevated. This may be due to their body's normal mechanism and indicates that no abnormality occurred to them because they showed no reduction. On the 4th blood collection, *A. spinosus* was administered to test group for another 7 days making a total of 14 days of treatment. The treated group had a slight elevation in the number of platelets(556.25 to 677.5) while the bleeding (82.5 to 60) and clotting time (45 to 31.75) slighty decreased. This slight elevation may indicate the that the effect of *A. spinosus* is still present but not as convincing as the values on the 7th day. This showed that the increasing effect of the plant was only maybe to normalize the platelet count but not to continually increase it which can prevent thrombocytosis. Positive control group (550 to 503) showed reduction on their platelet count which can indicate that the slight elevation during the 3rd blood collection is not constant. This may mean that the natural response of the body can't be solely relied on when reduction on the platelet count had occured. Vehicle and time control group values also showed a slight elevation in the platelet count and varries in the bleeding and clotting time which may still indicate no abnormality.

A.Effect of *A. spinosus*on Platelet Count

After the induction of ethanol, platelet counts were significantly decreased ($p<0.05$) in test and positive control groups($p=0.004$ and $p=0.031$ respectively) as seen in Table 2. This reduction on the number of platelets tallies to the altered platelet distribution by ethanol which caused the development of thrombocytopenia (Apostol et al.,2012). The ethanol caused the portal hypertension and hypersplenism which reduced the platelet count. The decreasing numbers of platelets available in the periphery were caused by these two factors which are due to the sequestration and accumulation of platelets in the spleen (Kakar, Kamath & Burgart, 2004). Furthermore, liver damage by ethanol can lessen the production

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of thrombopoetin which decreases platelet production (Salem & Laposata , 2005; Apostol et al., 2012).

Table 2
Significance of the platelet count of each groups during the different phases of the experiment using pair sample t- test

	p- value	Interpretation
Group A		
Pair 1	0.004	Significant
Pair 2	0.01	Significant
Pair 3	0.166	Not Significant
Group B		
Pair 1	0.031	Significant
Pair 2	0.062	Not Significant
Pair 3	0.538	Not Significant
Group C		
Pair 1	0.804	Not Significant
Pair 2	0.059	Not Significant
Pair 3	0.12	Not Significant
Group D		
Pair 1	0.961	Not Significant
Pair 2	0.553	Not Significant
Pair 3	0.081	Not Significant

Significant Difference: $P<0.05$

Pair 1- Before induction of ethanol (Baseline)
7 days after ethanol induction

Pair 2- 7 days after ethanol induction
A. spinosus administration for 7 days

Pair 3- *A. spinosus* administration for 7 days
A. spinosus administration for 14 days

After the 7 days administration of *A. spinosus*, the platelet count significantly increased (p=0.01) presented in Table 2. Continuous administration of the treatment until the 14th day showed no significant difference (p=0.166) in the platelet count from the 7th day treatment . This specifies that the platelet count elevation is constant and no reduction in the platelet count were seen. In positive control group, evaluation between 7 days after ethanol induction and after 7 days of no treatment showed that there is no clinical significant (p=0.062) which was caused by the destruction of the liver from ethanol induction. Liver is the principal organ responsible for the sensitization of thrombopoietin which regulate the production of platelets (Kujovich, 2005). In the 2nd week after ethanol induction there was still no clinical significant (p=0.538) which indicates that the slight increase in the mean of positive control group was not statistically significant. Vehicle control group had no significant difference on their platelet counts

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because it served as the vehicle control during the time of induction of ethanol before and after in test group (p=0.804), after giving of equal amount of distilled water for 7 days (p=0.059) and comparison of 7th day and 14th day after continuous administration of distilled water (p=0.12). This means that distilled water which was induced can't significantly increase or cause any major change in the platelet count. Time control group also had no clinical significance because it served as the time control group. It had a p value of 0.961 before and after the induction of ethanol, (p=0.553) after induction and administration of *A. spinosus* in test group, and a p value of 0.081 after the 14th day administration of treatment in the same group. This indicates that time control group showed no significant change on their platelet counts because it did not receive any treatment during the whole experiment phase.

B. Effect of *A. spinosus* on Clotting Time

Clotting time evaluates coagulation and the ability of clotting factors to form blood clots at the site of injury along with the degree of activation of the coagulaton pathways (Apostol et al., 2012).

Table 3

Significance of the clotting time of each groups during the different phases of the experiment using pair sample t- test

	p-value	Interpretation
Group A		
Pair 1	0.029	Significant
Pair 2	0.034	Significant
Pair 3	0.134	Not Significant
Group B		
Pair 1	0.001	Significant
Pair 2	0.119	Not Significant
Pair 3	0.426	Not Significant
Group C		
Pair 1	0.407	Not Significant
Pair 2	0.958	Not Significant
Pair 3	0.83	Not Significant
Group D		
Pair 1	0.843	Not Significant
Pair 2	0.875	Not Significant
Pair 3	0.391	Not Significant

Significant Difference: P<0.05

Pair 1- Before induction of ethanol (Baseline)
7 days after ethanol induction

Pair 2- 7 days after ethanol induction
A. spinosus administration for 7 days

Pair 3- *A. spinosus* administration for 7 days
A. spinosus administration for 14 days

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Table 3 shows the results of the clotting time of all the four groups during the different phases of the experiment. Pair 1 of test and positive control groups, correlation between the baseline bleeding time and ethanol induction, showed significance (p=0.029 and p=0.001 respectively). This was probably due to the induction of ethanol which induced thrombocytopenia resulting in the increase of their bleeding time. Vehicle and time control groups showed no significance (p=0.407 and p=0.843). The said groups functioned as a control so they were not given any ethanol. Thrombocytopenia was not induced in both groups. Pair 2 of test group still showed significance (p-value=0.034). This group underwent the A.spinosus treatment for 7 days. Bleeding time was reduced and improved. The positive control group, showed no significance (p-value=0.119). They were not given *A. spinosus* as a treatment for the ethanol-induced thrombocytopenia. Vehicle and time control groups still showed no significance (0.958 and 0.875) which appeared to be normal to occur. Pair 3 of test group did not show significance (p-value=0.134). *A.spinosus* was not administered during this period.This indicates that the clotting time did not statistically change which correlates with the platelet count that also showed no significance on the 14th day of *A. spinosus*
treatment. Positive, vehicle and time control groups continued to show no significance (p=0.426, p=0.83, and p=0.391 respectively) because no treatment were given to them during this time of the experiment phase.

C. Effect of *A. spinosus* on Bleeding Time

Bleeding time is a factor used to evaluate platelet aggregation and detect qualitative defects of platelets. Haemostatic function is usually assessed through the use of Duke's method as a reflection of haemorrhagic potential (Apostol et al., 2012). Possible disposition to prolonged hemorrhage and excessive blood loss may arise due to the increased bleeding time. The increase in bleeding time is inversely proportional to the number of platelets in circulation and correlates with the previously discuss section.

Upon the induction of ethanol, the bleeding time of test group (p=0.08) and positive control group(0.08) was not significantly increased in terms of statistical analysis presented in Table 4 but there is a slight elevation in the values displayedby test group (90 to 127.5) and positive control group (157.5 to 232.5) as seen in Table 1.

Tail vein bleeding in mice is currently the most generally used bleeding challenge for assessment of haemostatic state. The absence of significant increase may be caused by other factors like the vasoconstrictive effect of blood vessels as proven by Elderbi, Hadi & Mohamed, 2010. Though standardized, the test may, in the end, be

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unable to identify very essential bleeding disorders, because unstable clots in the presence of vascular spasm may be sufficient to temporarily protect against bleeding (Greene, Schiviz, Hoellriegl, Poncz, & Muchitsch, 2010).

Table 4
Significance of the bleeding time of each groups during the different phases of the experiment using pair sample t- test

	p-value	Interpretation
Group A		
Pair 1	0.08	Not Significant
Pair 2	0.014	Significant
Pair 3	0.391	Not Significant
Group B		
Pair 1	0.08	Not Significant
Pair 2	0.08	Not Significant
Pair 3	0.161	Not Significant
Group C		
Pair 1	0.664	Not Significant
Pair 2	0.824	Not Significant
Pair 3	0.718	Not Significant
Group D		
Pair 1	0.638	Not Significant
Pair 2	1	Not Significant
Pair 3	0.215	Not Significant

Significant Difference: $P<0.05$

Pair 1- Before induction of ethanol (Baseline)
7 days after ethanol induction

Pair 2- 7 days after ethanol induction
A. spinosus administration for 7 days

Pair 3- *A. spinosus* administration for 7 days

This statistical result may also be due to the fact that laboratory animals were stressed caused by the methods used for bleeding time, Duke's method. It was reported by various workers through experimental and clinical observations that psychological stress can alter the blood coagulation mechanism. Alteration of the blood coagulation system brought by the stress of surgery and emotions of fear and anxiety were shown by clinical reports (Mogenson & Jaques, 2013).Upon the 7th day induction of*A. spinosus*, the bleeding time of test group (p=0.014) significantly improved. This may be due to the elevation of platelets during this time. There was no significant difference on the bleeding time of the ethanol-induced thrombocytopenic rats treated with *A. spinosus*from the 7th day of therapy to the 14th day. This correlates to the no significant difference

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obtained in the platelet count and clotting time. The reduction of the bleeding time is vital in reducing the number and severity of bleeding episodes on patient suffering from hemorrhagic disorders (i.e. DHF) (Apostol et al., 2012).Positive control group (p=0.08 and p= 0.161) showed no significant difference during the 7th and 14th day of *A. spinosus* treatment to test group respectively. This may be due to the ethanol induced in this group which received no treatment of the plant decoction. There was no significant difference seen in vehicle (p=0.664, p=0.824, p=0.718) and time control groups (p=0.638, p=1, p=0.215) throughout the experiment phase for no major treatment was given to them.

III. Histopathological examination

Tissue examination of the liver and spleen supplied additional evidence of thrombocytopenia as induced by ethanol and the curative effects of the decoction. Liver damage, which can be due to chronic intake of ethanol, indicates an elevation in the blood pressure of the portal veins which will disrupt the flow of blood out of the spleen. This leads to the platelets being sequestered in the spleen. The dilation of the sinusoids of these organs are often the result of poor venous outflow (Kakar, Kamath & Burgart, 2004).The slides of the liver and spleen tissues were prepared by the Pathologists Diagnostic Center. They were then examined and analyzed by Dr. Joseph S. Masangkay of the Department of Veterinary Paraclinical Sciences in University of the Philippines, Los Baños.

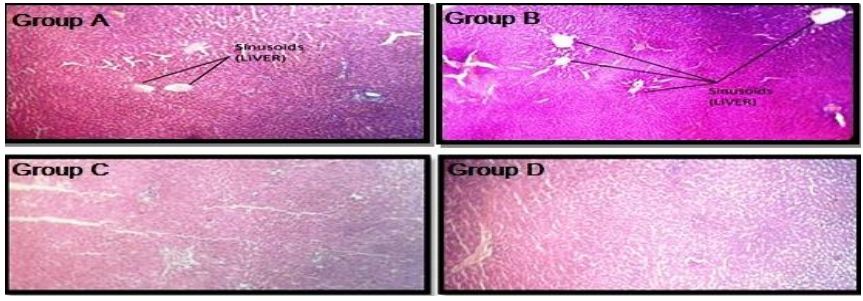

Figure 1. Representative images of the livers of Sprague Dawley rats in each groups. Group A (Test Group) Group B (Positive Control Group) Group C (Vehicle Control

Figure 1 presents the histopathological results of all the groups. Test group exhibits the liver sinusoids which showed slight dilatation with no other lesions observed in the hepatocytes. In positive control group, the liver sinusoids showed moderate dilatation with many hepatocytes showing pyknotic nuclei which are a prelude to necrosis which is due to the toxic effect of ethanol. While in the time and vehicle control groups which is showed in Groups C and D, the

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liver section showed no apparent lesion. This indicates that the liver of the two groups were normal maybe because no induction of ethanol had occurred to them.

No noteworthy change was observed in the spleen sinusoidal dilation of the three animal groups as presented in Figure 2. All three groups were graded with NAL(No apparent Lesion). The probable reason behind this result was the brief period of 1 week for the induction of thrombocytopenia (Apostol et al., 2012). During the said duration, liver damage may have been provoked but was still insufficient to cause any significant change to the spleen.

Upon the evaluation of the liver and spleen, indications of ethanol-induced thrombocytopenia was observed along with the therapeutic effects of *A.spinosus* decoction. The decoction of the said plantgiven to the experimental rats in test group apparently had a curative effect on the sinusoidal dilatation induced by ethanol. The decoction also had an apparent preventive effect in inducing hepatic necrosis which was seen in the liver of positive control group given ethanol. The presence of phenolic compounds in the plant might be responsible for its marked antioxidant activities in the liver damage caused by ethanol (Srinivasa et al., 2010).*A. spinosus*is considered to be safe for the treatment in liver problems at normal therapeutic doses (Gul, Srivastava, & Ahirwar, 2011).

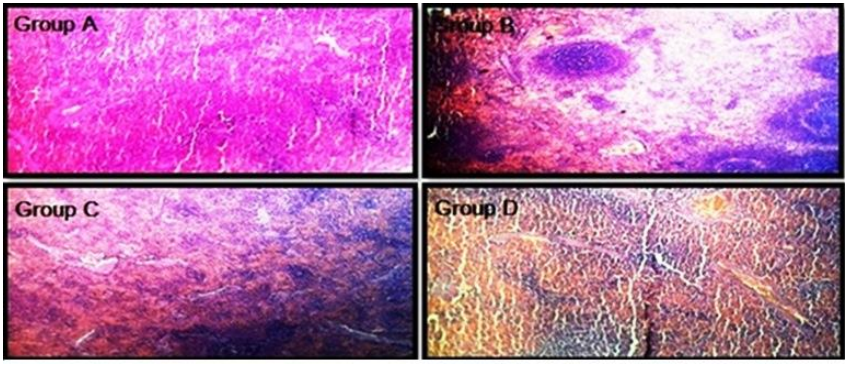

Figure 2: Comparison between the spleen tissues of all groups.Group A (Test Group) Group B (Positive Control Group); Group C (Vehicle Control Group) Group D (Time Control Group)

CONCLUSION

In conclusion, the presence of phenolic content was detected in *A. spinosus.* This phenolic content which is responsible for the anti ontioxidant acitvity may be accountable to the elevated platelet count and improved bleeding and clotting time of the rats induced with 100 mg/kg dosage of *A. spinosus* decoction. To validate these findings

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further, the liver histopathologic results also suggested that the decoction given had a therapeutic effect on the sinusoidal dilatation induced by ethanol and had an apprerent curative effect in inducing hepatic necrosis. This study encompasses the presumptive therapeutic effect of *A.spinosus* on ethanol-induced thrombocytopenic rats.

RECOMMENDATION

Further research must be conducted utilizing the active component of *A. spinosus*. The antithrombocytopenic activity of *A. spinosus* using varying concentrations must be performed.Clinical testing must also be conducted

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